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U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

218477US0PCT

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

10/030142

INTERNATIONAL APPLICATION NO.
PCT/JP00/05114INTERNATIONAL FILING DATE
28 JULY 2000PRIORITY DATE CLAIMED
29 JULY 1999

TITLE OF INVENTION

METHOD FOR TREATING FERTILIZED EGGS AND METHOD FOR HATCHING FERTILIZED EGGS

APPLICANT(S) FOR DO/EO/US

Yoshiyuki OHTA, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☒ has been communicated by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☒ is attached hereto.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Notice of Priority / PCT/IB/304 / PCT/IB/308
 PTO-1449

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

INTERNATIONAL APPLICATION NO.

ATTORNEY'S DOCKET NUMBER

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PCT/JP00/05114

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24. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

- ☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1040.00
- ☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$890.00
- ☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$740.00
- ☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00
- ☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =**CALCULATIONS PTO USE ONLY**

\$890.00

\$0.00

Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)).

☐ 20 ☐ 30

CLAIMS

NUMBER FILED

NUMBER EXTRA

RATE

Total claims

30 - 20 =

10

x \$18.00

\$180.00

Independent claims

3 - 3 =

0

x \$84.00

\$0.00

Multiple Dependent Claims (check if applicable).

☒

\$280.00

TOTAL OF ABOVE CALCULATIONS =

\$1,350.00

Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.

\$0.00

SUBTOTAL =

\$1,350.00

Processing fee of \$130.00 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)).

☐ 20 ☐ 30

+

\$0.00

TOTAL NATIONAL FEE =

\$1,350.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).

☐

\$0.00

TOTAL FEES ENCLOSED =

\$1,350.00

Amount to be:

refunded

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- a. ☒ A check in the amount of \$1,350.00 to cover the above fees is enclosed.
- b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 15-0030. A duplicate copy of this sheet is enclosed.
- d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:



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REGISTRATION NUMBER

DATE

Jan 28 2002

DESCRIPTION

METHOD FOR TREATING FERTILIZED EGGS AND

METHOD FOR HATCHING FERTILIZED EGGS

5

TECHNICAL FIELD

The present invention relates to an improvement of a method for producing chicks by artificial hatch of fertilized eggs of birds. More particularly, the present invention relates to a method for enhancing the productivity of chicks by carrying out a treatment for supplying nutrition by injecting a mixed solution of amino acids into fertilized eggs.

15 BACKGROUND OF THE INVENTION

In the factories of producing chicks including broilers, efforts for improvement have been made for enhancing the productivity of hatched chicks until they grow to a stage suitable for eating. For example, in chickens, it is tried to accelerate the growth by adding various nutrition-supplements such as vitamins to feeds.

Moreover, since the size of chicks hatched from fertilized eggs and the growth rate after hatch are generally proportional to the size and protein amount of fertilized eggs, much efforts for improving nutrition of parent chickens have been made for the purpose of producing

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fertilized eggs having a large size and containing a large
amount of proteins. However, an egg-production ratio is
generally low in the parent chickens laying fertilized eggs
having a large size and containing a large amount of
5 proteins. Therefore, the feeding cost for the parent
chickens cannot be disregarded, which is required for
securing regularly a large number of such fertilized eggs
having a large size and containing a large amount of
proteins.

10 DISCLOSURE OF THE INVENTION

An object of the present invention is to provide a
method capable of supplying regularly a large number of
chicks at a low cost, the chicks having body weight at
15 hatch heavier than that usually expectable from the size of
fertilized eggs and being capable of shortening the term
necessary for reaching a stage suitable for eating after
hatch. More specifically, an object of the present
invention is to provide a method for treating fertilized
20 eggs for obtaining fertilized eggs capable of supplying
chicks which have heavy body weight at hatch and are
capable of shortening the term necessary for reaching a
stage suitable for eating after hatch.

In order to achieve the above and other objects,
25 the present invention basically relates to injection of
amino acids into fertilized eggs of birds, the amino acids

being substances determining the nutritive value of the proteins in the fertilized eggs, and the present invention includes the following each invention.

(1) A method for treating a fertilized egg of a bird, comprising injecting, into the fertilized egg after start of incubation, a solution containing amino acids which determine the nutritive value of proteins in the fertilized egg.

(2) The method according to (1), wherein the fertilized egg is a chicken egg on days 13 to 19 of the incubation..

(3) The method according to (1), wherein the fertilized egg is a chicken egg on days 13 to 14 of the incubation.

(4) The method according to any one of (1) to (3), wherein the solution containing amino acids is a mixed solution of amino acids having a composition which is almost the same as the amino acid composition in the fertilized egg before the start of incubation.

(5) The method according to any one of (1) to (4), wherein the solution containing amino acids contains an antioxidant, a nutritional supplement other than said amino acids, and/or a vaccine.

(6) A method for hatching a fertilized egg of a bird, comprising hatching the fertilized egg after start of incubation treated according to the method of any one of (1) to (5).

(7) A method for increasing the body weight of a chick at hatch, comprising injecting, into a fertilized egg after start of incubation, a solution containing amino acids which determine the nutritive value of proteins in the fertilized egg.

(8) The method according to (7), wherein the fertilized egg is a chicken egg on days 13 to 19 of the incubation.

(9) The method according to (7), wherein the fertilized egg is a chicken egg on days 13 to 14 of the incubation.

(10) The method according to any one of (7) to (9), wherein the solution containing amino acids is a mixed solution of amino acids having a composition which is almost the same as the amino acid composition in the fertilized egg before the start of incubation.

(11) The method according to any one of (7) to (10), wherein the solution containing amino acids contains an antioxidant, a nutritional supplement other than said amino acids, and/or a vaccine.

(12) A method for increasing the hatchability of a fertilized egg of a bird, comprising injecting, into the fertilized egg after start of incubation, a solution containing amino acids which determine the nutritive value of proteins in the fertilized egg.

(13) The method according to (12), wherein the fertilized egg is a chicken egg on days 13 to 19 of the incubation.

(14) The method according to (12), wherein the fertilized egg is a chicken egg on days 13 to 14 of the incubation.

(15) The method according to any one of (12) to (14),
5 wherein the solution containing amino acids is a mixed solution of amino acids having a composition which is almost the same as the amino acid composition in the fertilized eggs before the start of incubation.

(16) The method according to any one of (12) to (15),
10 wherein the solution containing amino acids contains an antioxidant, a nutritional supplement other than said amino acids, and/or a vaccine.

(17) A fertilized egg of a bird after start of incubation, said egg being treated according to the method
15 of any one of (1) to (5).

(18) A bird hatched from a fertilized egg after start of incubation, said egg being treated in accordance with the method according to any one of (1) to (5).

20 BEST MODE FOR CARRYING OUT THE INVENTION

The fertilized eggs to be treated by the method of the present invention are not particularly limited, so long as the eggs are eggs of birds capable of subjecting to artificial hatch. The injection of nutrient into
25 fertilized eggs by the above treatment of injecting amino acids improves not only the growth of embryos in the

fertilized eggs after the start of incubation but also the growth of chicks hatched from the treated fertilized eggs. Therefore, the treating method of the present invention is particularly suitable for the treatment of fertilized eggs
5 of chickens including broilers.

The composition of the solution containing amino acids for use in the method of the present invention is not particularly limited as far as the solution contains amino acids determining the proteins in fertilized eggs.
10 Preferred is a mixed solution of amino acids having a composition which is almost the same as the amino acid composition in the fertilized eggs before the start of incubation. Hereinafter, the solution containing amino acids for use in the method of the present invention is
15 abbreviated as an "amino acid solution".

Examples of a suitable amino acid solution include an amino acid solution having a composition shown in Table 1.

In the amino acid composition in Table 1, it is
20 possible to use phenylalanine instead of tyrosine. Moreover, any mixed solution of amino acids having the composition ratio of amino acids shown in Table 1 can be employed even when the concentration is different.

Table 1
(Composition of Amino Acids)

Amino acid	(g/l)
Asparagine	10.6±1
Threonine	5.0±1
Serine	7.7±1
Glutamine	14.0±1
Glycine	3.5±1
Alanine	6.0±1
Valine	6.7±1
Cystine	2.2±1
Methionine	3.8±1
Isoleucine	5.4±1
Leucine	9.1±1
Tyrosine	1.7±1
Phenylalanine	5.6±1
Lysine	7.6±1
Histidine	2.7±1
Arginine	6.5±1
Proline	3.9±1
Tryptophan	1.9±1

In the present invention, the amino acids are
5 injected as a solution in a liquid, and the liquid to be
used is not particularly limited unless it adversely
affects the incubation. Examples include distilled water
and physiological saline.

The concentration of the amino acid solution for
10 use in the method of the present invention and the amount
of amino acids to be injected are not particularly limited,
so long as necessary amounts of amino acids can be

administered into fertilized eggs. The amino acid solution can be used in a concentration range of usually 30 to 220 g/l, preferably 30 to 180 g/l, and more preferably 50 to 160 g/l.

Moreover, the amount of the amino acid solution to be injected into fertilized eggs is an amount capable of administering a necessary amount of aimed amino acids into the fertilized eggs, and the amount to be injected generally ranges from 0.2 to 1.0 ml/fertilized egg.

Furthermore, the amino acid solution may contain an antioxidant, a nutritional supplement such as a vitamin, and other additives and carriers which do not affect adversely on the hatch of fertilized eggs and the growth of birds.

The treatment of injecting the amino acid solution into fertilized eggs by the method of the present invention is subjected to the fertilized eggs after the start of incubation. The part of injecting the amino acid solution into the fertilized eggs after the start of incubation is the parts of egg yolk and albumen, and/or inner tissues of fertilized eggs, excluding an air cell and an embryo in the fertilized eggs. At the part of the air cell, the amino acid solution is blocked by chorioallantois and thus no nutrition-supplying effect on the embryo is realized, while there is a possibility that the embryo is destroyed by entering the needle for injection to cause no occurrence of

hatch in injection into the embryo part. Therefore, it is preferable to avoid the injection into these parts.

The timing for injection is preferably the time when the embryo rapidly grow in the fertilized eggs.

5 In chicken eggs, for example, with the growth of
the embryo in a fertilized egg, the egg yolk in saccus
vitellinus gradually decreases until about days 7 of the
incubation, but rapid decrease of the egg yolk is observed
10 on day 7 to about day 14, and then after day 14, the
decrease of the yolk becomes again gradual. From these
facts, as the timing for nutrition enhancement of
incubating eggs by injecting amino acids, the injection is
effective on days 7 to 14 of the incubation. Particularly,
the injection of amino acids is carried out on days 10 to
15 14, more preferably on days 13 to 14, and particular
preferably on day 14 of the incubation. After day 14, the
effect of injection is not so remarkable as compared with
the injection until day 14, but the effects of improving
hatchability of fertilized eggs and body weight at the
20 hatch by the injection of the amino acid solution can be
expected until day 19 depending on the kind of fertilized
eggs.

Moreover, especially on days 10 to 14 of the
incubation, the embryo is resistant to the amino acid
25 solution even when the amino acid solution comes into
contact with the growing embryo and the embryo is less

damaged owing to low possibility of the contact with the
needle, so that the part to be injected is not particularly
limited. Accordingly, the injection is preferably carried
out during this term also from the viewpoint of the
5 easiness of injection operation. When amino acids are
injected on days 7 to 9 of the incubation, the injection
into the egg yolk part is preferable.

In fertilized chicken eggs, the parts of the egg
yolk and albumen in the fertilized eggs have regions
10 capable of easily inserting a needle for injection into the
parts until about day 14 after the start of incubation and
the embryo itself is resistant so as not to be damaged
easily by the needle for injection, so that the injection
of the amino acid solution is preferably carried out on day
15 14. As above, the injection of the amino acid solution at
the time when the embryo is resistant to the damage by the
needle for injection, the growth of the embryo is rapid and
nutrition consumption is fast provides an important
technical significance that the treatment of injecting
20 amino acids can be realized by means of an automatic
apparatus which injects the amino acid solution into a
large number of fertilized eggs at once with a large number
of needles for injection arranged in a line. However, so
long as a careful injection of amino acids is carried out
25 so as not to damage the tissues in the fertilized eggs, the
effects of the amino acid injection can be fully expected

even after day 14. In addition, the amino acid solution can be injected together with a vaccine such as the vaccine for Marek's disease.

5 The treatment of injecting amino acids according to the present invention can increase the body weight of chicks, for example, at hatch by 1 to 2% as the ratio of body weight at hatch/egg weight. Moreover, the hatchability can be increased, for example, by 5 to 30%.

10 EXAMPLES

The present invention is more specifically explained with reference to Examples, but the present invention is not limited to these Examples.

15 Example 1

Fertilized eggs of a broiler (Chunky) were sterilized with 70% alcohol and the eggs were divided into 6 groups, each group being composed of 10 eggs, based on the measurement of egg weight so that each group had the same average egg weight±standard deviation (51.2±0.2 g), followed by incubation at a temperature of 37.8°C and a relative humidity of 60%. The eggs under incubation were checked every one week to remove undeveloped eggs. On day 14 of the incubation, one group was left untreated as a control group, and a hole was made at the eggshell on the air cell of each egg of remaining five groups, followed by

20

25

injection of 0.5 ml of a solution (hereinafter, referred to as "amino acid solution") of an amino acid mixture dissolved in sterile distilled water into the egg yolk, the mixture being used in an amount so as to become a concentration of 0, 53, 106, 159, or 212 mg/ml. Thereafter, the hole was closed with paraffin and the incubation was continued. The injection treatment was carried out aseptically in a clean bench.

The amino acid solution used for the injection had a composition similar to the composition shown in Table 1, with the exception that sparingly soluble tyrosine was replaced by phenylalanine.

With regard to the fertilized eggs, the hatching time and the body weight at hatch were measured every one hour from day 19 of the incubation, the starting day of the incubation being zero day.

A 1 ml disposable syringe (manufactured by Terumo Corporation) was used as the syringe for injecting the amino acid solution into the fertilized eggs and 27Gx3/4" (manufactured by Terumo Corporation) was used as the needle for injection.

With regard to the hatchability, a significant difference was determined by a χ -square test based on the results obtained. With regard to other indexes, analysis of variance was carried out and difference between average

values was tested by LSD method. The statistical risk was set to 5%.

The results are shown in Table 2.

5

Table 2

Treated group	Egg weight (g)	Hatch-ability (%)	Body weight at hatch (g)	Body weight at hatch/egg weight (%)
Control Group	51.0±2.9 ¹⁾	70.0 ¹⁾	36.9±3.2 ¹⁾	73.0±2.6 ¹⁾
Amino acidinjected Groups (mg/ml)				
53.0	51.1±2.0	90.0	36.5±4.5	71.8±7.6
106.0	51.5±2.2	90.0	38.6±1.9	75.0±1.3
159.0	51.4±2.4	80.0	38.0±2.5	73.5±2.1

1) Each value is an average value±standard deviation of 10 fertilized eggs.

Table 2 shows that the hatchability of the fertilized eggs of control group is 70.0%, but the hatchability of the fertilized eggs to which the amino acid solution having a concentration of 53.0 mg/ml to 159.0 mg/ml was injected is from 80 to 90%. This result apparently indicates that the method of the present invention contributes the enhancement of hatchability of fertilized eggs.

Moreover, with regard to the body weight at hatch, the body weight at hatch/egg weight of the fertilized eggs of the control group is 73.0±2.6%, while the ratio of the fertilized eggs to which the amino acid solution having a

concentration of 53.0 mg/ml to 159.0 mg/ml was injected is the same as or higher than the value. In particular, the group injected with an amino acid solution having a concentration of 106.0 mg/ml shows a value of $75.0 \pm 1.3\%$,
5 which clearly indicates that the injection of the amino acid solution is a method capable of obtaining chicks having heavier body weight at hatch than those of non-treated fertilized eggs.

10 Example 2

Fertilized eggs of a broiler (Chunky) were sterilized with 70% alcohol and the eggs were divided into 6 groups, each group being composed of 10 eggs, based on the measurement of egg weight so that each group has the
15 same average egg weight \pm standard deviation (51.2 ± 0.2 g), followed by incubation at a temperature of 37.8°C and a relative humidity of 60%. The eggs under incubation were checked every one week to remove undeveloped eggs. Day 14
20 of the incubation, one group was left untreated as a control group, and a hole was made at the eggshell on the air cell of each egg of remaining five groups, followed by injection of an amino acid solution having a concentration of 106 mg/ml in an amount of five different levels, i.e., 0, 0.25, 0.5, 0.75, and 1 ml into the egg yolk. Thereafter,
25 the hole was closed with paraffin and the incubation was

continued. The injection treatment was carried out aseptically in a clean bench.

The amino acid solution used for the injection had a composition similar to the composition used in above
5 Example 1, and the syringes and needles for injecting the amino acid solution into the fertilized eggs were also the same as those used in Example 1.

With regard to the fertilized eggs, the hatching time and the body weight at the hatch were measured every
10 one hour from day 19 of the incubation, the starting day of the incubation being zero day.

With regard to the hatchability, a significant difference was determined by a χ -square test based on the results obtained. With regard to other indexes, analysis
15 of variance was performed and difference between average values was tested by LSD method. The statistical risk was set to 5%.

The results are shown in Table 3.

Table 3

Injected amino acid amount (ml)	Egg weight (g)	Hatch-ability (%)	Body weight at hatch (g)	Body weight at hatch/egg weight (%)
0 (Control)	58.3±2.6 ¹⁾	75.0 ¹⁾	42.6±2.6 ^{b1)}	73.7±2.6 ^{b1)}
0.25	58.3±2.5	90.0	43.7±2.3 ^{ab}	75.3±2.7 ^{ab}
0.5	58.3±2.5	85.0	43.8±2.5 ^{ab}	75.4±3.7 ^{ab}
0.75	58.3±2.5	75.0	44.5±2.3 ^a	76.4±3.3 ^a
1.0	58.3±2.5	70.0	43.5±2.5 ^{ab}	74.8±3.3 ^{ab}
0.5 (212 mg/ml)	58.3±2.5	70.0	43.4±2.2 ^{ab}	74.3±1.8 ^{ab}

1) Each value is an average value±standard deviation of 20 fertilized eggs.

a,b: There is a significant difference between different symbols by LSD method ($P<0.05$).

Table 3 shows that the hatchability of the fertilized eggs of the control group is 75.0%, but the hatchability of the fertilized eggs to which 0.25 ml or 0.5 ml of the amino acid solution having a concentration of 106.0 mg/ml was injected is from 85 to 90%. Moreover, with regard to the body weight at hatch/egg weight (%), the body weight at hatch/egg weight of the fertilized eggs of the control group is 73.0±2.6%, while the ratios of the fertilized eggs in the two cases were 75.3±2.7% and 75.4±3.7%, respectively, these values indicating a significant difference from the control. Furthermore, when the injected amount of the amino acid solution was 1.0 ml and when 0.5 ml of the amino acid solution having doubled concentration was injected so that the injected amount of amino acids is the same, the hatchability is the same, i.e.,

70.0% which is inferior to the value of the control group,
but the body weight at hatch/egg weight (%) is $74.8 \pm 3.3\%$
and $74.3 \pm 1.8\%$, while the value of the control group is
 $73.7 \pm 2.6\%$. Thus, the significant difference can be
5 confirmed.

Example 3

Influence of the injection of amino acid into broiler
fertilized eggs at later stage of incubation on
10 hatchability and body weight at hatch:

Fertilized eggs of Cobb broiler were divided into
seven groups, each group being composed of 20 eggs.

In order to examine the influence of the injection
of amino acids at later stage of incubation on hatchability,
15 seven groups were set, one group being a control and amino
acids being injected into the eggs of each one of the
remaining six groups at each one day of from day 14 of the
incubation to day 19 which is immediately before hatch.
Fertilized eggs having an average egg weight of 68 g were
20 used and incubated at a temperature of 37.8°C and a
relative humidity of 60%. Specifically, the following
groups were set: a control group; and seven groups in total
wherein 0.5 ml of 106 mg/ml of an amino acid solution
having the protein composition which is the same as that of
25 the whole egg proteins was injected into each of the
fertilized eggs on days 14, 15, 16, 17, 18 and 19 of the

incubation. A 1 ml syringe for tuberculinization and a needle of 27Gx3/4' were used for the injection of amino acids.

Hatchability and body weights at hatch were measured. The results obtained were subjected to a χ -square test to compare the hatchability. The other results were subjected to analysis of variance.

Since fertilized eggs exhibiting very high hatchability were used, the hatchability in the control group was found to be a high rate, i.e., 96.4%, and the injected group on day 14 of the incubation resulted in a similar high hatchability (Table 4). However, the hatchability decreased in the groups wherein the injection was carried out thereafter. The reason why the hatchability decreased is considered that the amino acid solution was injected into the air cell because the needles used for the injection were short. Only one example of no hatch because of the insertion of the needle into embryo was observed among the injected group injected on day 19 of the incubation. From these results, there is a possibility of overcoming the problem of hatchability by investigating the conditions for injecting amino acids and the problem is not significant when the injection of the vaccine for Marek's disease is considered.

Table 4

Treated day	Egg numbers	Initial egg weight (g)	Hatched egg weight (g)	Hatch-ability (%)	Body weight at hatch (g)	Body weight at hatch/egg weight (%)
Control	29	65.6±4.8	65.6±5.0	96.6	49.1±3.5	74.9±2.5 ^b
14	33	65.7±4.8	65.8±5.0	96.9	50.0±3.5	76.1±2.1 ^{ab}
15	31	67.9±4.8	65.9±4.4	93.5	50.1±3.3	76.2±5.3 ^{ab}
16	31	66.6±4.8	65.9±4.8	93.5	50.7±3.2	77.1±1.6 ^{ab}
17	28	65.7±4.8	65.9±5.0	89.3	49.6±3.3	75.4±1.2 ^{ab}
18	32	65.7±4.8	65.4±4.5	87.5	49.2±3.7	75.2±3.0 ^b
19	32	65.7±4.8	65.7±5.0	90.6	49.6±4.6	75.5±4.5 ^{ab}

a,b: There is a significant difference between different symbols.

The values are shown by average ± standard deviation.

Hatchability is calculated excluding unfertilized eggs.

5

Example 4

Influence of the injection of amino acid into fertilized eggs of different kind of broilers at later stage of incubation on hatchability and body weight at hatch:

10

Two hundred fertilized eggs of Cobb and Arbor Acres broilers were purchased and the eggs were divided into 2 groups per each kind of chickens, each group being composed of 50 eggs, so that each group had the same average egg weight (65.0±4.0 g and 64.9±4.2 g; average egg

15

weight±standard deviation). One group was a control group. With regard to the fertilized eggs, the starting day of the incubation was zero day, and a group to which an amino acid solution was injected was set.

Incubation was carried out at a temperature of 37.8°C and a relative humidity of 60%. The eggs under incubation were checked every one week to remove undeveloped eggs.

5 Day 19 of the incubation, all the fertilized eggs were taken out of the incubator, and a hole was made at the eggshell on the air cell of each egg of the group to be injected with amino acids, followed by injection of 0.5 ml of a solution (hereinafter, referred to as an "amino acid
10 solution") of an amino acid mixture dissolved in sterile distilled water into the egg yolk, the mixture being used in an amount so as to become a concentration of 106 mg/ml. Thereafter, the hole was closed with paraffin, the eggs were returned to the incubator with in 1 hour from the
15 taken-out, and the incubation was continued. The injection treatment was carried out aseptically in a clean bench.

After hatching, the hatching time and the body weight at the hatch were measured.

The amino acid solution used for the injection had
20 a composition similar to the composition shown in Table 1 with the exception that sparingly soluble tyrosine was replaced by phenylalanine. A 1 ml disposable syringe (manufactured by Terumo Corporation) was used as the syringe for injecting the amino acid solution into the
25 fertilized eggs and 27Gx3/4" (manufactured by Terumo Corporation) was used as the needle.

With regard to the hatchability, a significant difference was determined by a χ -square test based on the results obtained. With regard to other indexes, analysis of variance was performed. The statistical risk was set to 5 5%.

As shown in Table 5, the hatchability of the fertilized eggs of Arbor Acres broiler is 87.8% in the amino acid-injected group, which is almost the same as 87.4% in the control group, so that no difference between 10 them was observed. Moreover, in the case of the fertilized eggs of Cobb broiler, the hatchability is 86.7% in the amino acid-injected group, which is slightly higher than 84.4% in the control group.

The ratio of the body weight at hatch/egg weight is 15 significantly increased by the injection of amino acids irrespective of the difference of kind of chickens.

Table 5

Line	Group	Egg numbers	Initial egg weight (g)	Hatched egg weight (g)	Hatchability (%)	Body weight of chick at hatch (g)	Body weight at hatch/egg weight (%)
Arbor Acres	control	48	64.9±4.8	65.0±4.3	87.4	48.0±3.4	74.0±0.6
	injected	49	64.9±4.8	64.8±4.0	87.8	48.4±3.8	74.7±1.4
Cobb	control	45	64.9±4.8	64.7±3.0	84.4	47.0±3.0	72.6±0.7
	injected	45	65.0±4.8	65.1±4.3	86.7	48.1±3.8	73.8±1.5
P<	line		NS	NS		NS	0.05
	injected		NS	NS		NS	0.05
	inter-action	NS	NS		NS	NS	

The values are shown by average ± standard deviation.

Hatchability is calculated excluding unfertilized eggs.

NS = no significant difference

5

INDUSTRIAL APPLICABILITY

According to the present invention, one or all of the purposes of improving hatchability of fertilized eggs and enhancing body weight at hatch/egg weight (%) can be accomplished by appropriately selecting the combination of the concentration and amount of an amino acid solution to be used in the method of injecting amino acids into fertilized eggs at a suitable stage after the start of incubation.

CLAIMS

1. A method for treating a fertilized egg of a bird, comprising injecting, into the fertilized egg after
5 start of incubation, a solution containing amino acids which determine the nutritive value of proteins in the fertilized egg.

2. The method according to claim 1, wherein the
10 fertilized egg is a chicken egg on days 13 to 19 of the incubation.

3. The method according to claim 1, wherein the
15 fertilized egg is a chicken egg on days 13 to 14 of the incubation.

4. The method according to any one of claims 1 to 3, wherein the solution containing amino acids is a mixed solution of amino acids having a composition which is
20 almost the same as the amino acid composition in the fertilized egg before the start of incubation.

5. The method according to any one of claims 1 to 4, wherein the solution containing amino acids contains an
25 antioxidant, a nutritional supplement other than said amino acids, and/or a vaccine.

6. A method for hatching a fertilized egg of a bird, comprising hatching the fertilized egg after start of incubation treated according to the method of any one of
5 claims 1 to 5.

7. A method for increasing the body weight of a chick at hatch, comprising injecting, into a fertilized egg after start of incubation, a solution containing amino
10 acids which determine the nutritive value of proteins in the fertilized egg.

8. The method according to claim 7, wherein the fertilized egg is a chicken egg on days 13 to 19 of the
15 incubation.

9. The method according to claim 1, wherein the fertilized egg is a chicken egg on days 13 to 14 of the incubation.
20

10. The method according to any one of claims 7 to 9, wherein the solution containing amino acids is a mixed solution of amino acids having a composition which is almost the same as the amino acid composition in the
25 fertilized egg before the start of incubation.

11. The method according to any one of claims 7 to 10, wherein the solution containing amino acids contains an antioxidant, a nutritional supplement other than said amino acids, and/or a vaccine.

5

12. A method for increasing the hatchability of a fertilized egg of a bird, comprising injecting, into the fertilized egg after start of incubation, a solution containing amino acids which determine the nutritive value of proteins in the fertilized egg.

10

13. The method according to claim 12, wherein the fertilized egg is a chicken egg on days 13 to 19 of the incubation.

15

14. The method according to claim 12, wherein the fertilized egg is a chicken egg on days 13 to 14 of the incubation.

20

15. The method according to any one of claims 12 to 14, wherein the solution containing amino acids is a mixed solution of amino acids having a composition which is almost the same as the amino acid composition in the fertilized eggs before the start of incubation.

25

16. The method according to any one of claims 12 to 15, wherein the solution containing amino acids contains an antioxidant, a nutritional supplement other than said amino acids, and/or a vaccine.

5

17. A fertilized egg of a bird after start of incubation, said egg being treated according to the method of any one of claims 1 to 5.

10

18. A bird hatched from a fertilized egg after start of incubation, said egg being treated in accordance with the method according to any one of claims 1 to 5.

Declaration and Power of Attorney For Patent Application

特許出願宣言書及び委任状

Japanese Language Declaration

日本語宣言書

下記の氏名の発明者として、私は以下の通り宣言します。

私の住所、私書箱、国籍は下記の私の氏名の後に記載された通りです。

下記の名称の発明に関して請求範囲に記載され、特許出願している発明内容について、私が最初かつ唯一の発明者（下記の氏名が一つの場合）もしくは最初かつ共同発明者（下記の名称が複数の場合）であると信じています。

上記発明の明細書は、

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- ☐ ____月____日に提出され、米国出願番号または特許協定条約国際出願番号を____とし、
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As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled.

METHOD FOR TREATING FERTILIZED EGGS AND

METHOD FOR HATCHING FERTILIZED EGGS

the specification of which

☐ is attached hereto.

☒ was filed on July 28, 2000

as United States Application Number or

PCT International Application Number

PCT/JP00/05114 and was amended on

____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

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私は、米国法典第35編119条 (a) - (d) 項又は365条 (b) 項に基づき下記の、米国外の国の少なくとも一カ国を指定している特許協力条約365 (a) 項に基づく国際出願、又は外国での特許出願もしくは発明者証の出願についての外国優先権をここに主張するとともに、優先権を主張している、本出願の前に出願された特許または発明者証の外国出願を以下に、枠内をマークすることで、示しています。

Prior Foreign Application(s)
外国での先行出願

P. He1. 11-214496	Japan
(Number) (番号)	(Country) (国名)
(Number) (番号)	(Country) (国名)

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私は、私自信の知識に基づいて本宣言書中で私が行なう表明が真実であり、かつ私の入手した情報と私の信じることに基づく表明が全て真実であると信じていること、さらに故意になされた虚偽の表明及びそれと同等の行為は米国法典第18編第1001条に基づき、罰金または拘禁、もしくはその両方により処罰されること、そしてそのような故意による虚偽の声明を行なえば、出願した、又は既に許可された特許の有効性が失われることを認識し、よってここに上記のごとく宣誓を致します。

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29/July/1999	Priority Claimed 優先権主張
(Day/Month/Year Filed) (出願年月日)	<input checked="" type="checkbox"/> Yes はい
(Day/Month/Year Filed) (出願年月日)	<input type="checkbox"/> No いいえ

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Japanese Language Declaration

(日本語宣言書)

委任状：私は下記の発明者として、本出願に関する一切の手続きを米特許商標局に対して遂行する弁理士または代理人として、下記の者を指名いたします。

(弁理士、または代理人の指名及び登録番号を明記のこと)

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